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Isolation and Identification of Aspergillus Species from Soil Sample, Hindu College, Guntur

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ABSTRACT

Aspergillus species are utilized in the fermentation industry. The investigation is aimed to find out the fungal diversity, also to isolate and identify the *Aspergillus* species in the soil samples collected in Hindu College campus, Guntur. For this isolation and identification, plating, pure culturing, incubating, staining, inoculating such techniques are used. The mycelia and cytoplasm are stained using Lactophenol and cotton blue (provides light blue background). The stained specimens are observed under the microscope for identification and photographs are taken. Among the identified species the Keratinophilic fungi *Aspergillus Niger* is found in maximum numbers in the campus and followed by others. All the isolates are identified with standard key and microbial expert.

Keywords: Aspergillus; Fungi; Isolation; Keratinophilic; Magnification; Mycelium.

1. Introduction

The microorganisms play a major role in soil ecosystem is explained by Ananthanarayana (2016) in his work Textbook of Microbiology. Microbial composition and functioning, changes the soil quality through decomposition of organic matter, recycling of nutrients and biological control. Soil is an oligotrophic medium for the growth of fungi because the fungal growths are extremely limited for most of the time and readily available. For most of the time,fungi are either dormant, or they metabolize and grow very slowly utilizing a range of organic molecules. The fungi distribute organic matter away from the roots. In general, the concentration of microbes is greatest close to the surface of roots (rhizosphere) and hyphae of arbuscular mycorrhizal fungi (mycorrhizosphere), where exudates are extraordinarily important source of organic energy entering from soils is explained by Surinder Kumar (2012). Fungi use antagonism to reduce competition by producing antibodies, which suppress other microorganisms from growing is explained by David Greenwood and Mike Barer (2007). They produce many vitamins which promote plant growth. Beneficial fungi also form protective webs and nets around roots and leaves to protect the host plant. Fungus also protects plants by supplying both water and phosphorus to the plant roots during droughts. Different fungal communities are identified from soil samples collected from different locations of Hindu college campus and it is identified and confirmed with microbial expert. Aspergillus are common moulds which are widespread and ubiquitous in nature. Aspergillus belong to the family *Trichocomaceae*. Aspergillus has a filamentous structure. (1) Isolation, screening and identification of fungi from soil by Rakesh Kumar Soni and Kavitha Sharma (2014), (2) Isolation, identification and seasonal distribution of penicillium and aspergillus species in Dal Lake, Kashmir by Suhaib et al. (2011), (3) Isolation and identification of fungi from soil in Loyola college campus, Chennai, India by Raja et al. (2017), (4) Isolation and identification of aspergillus species during one year in the hospitals by Iskendar Karalti and Gunay Tulay Colakgolu (2012), (5) Isolation and identification, screening and optimization of pectinase producing soil fungi (*A. niger*) by Kamalambigeswari and Ushani (2018), have isolated and identified Aspergillus in different locations, by implementing various techniques.

Here an attempt is made to isolate and identify the aspergillus species in the soil sample by employing different techniques (serial dilution, media preparation, plating, incubating). Results are confirmed by colony morphology and microscopical observation.

2. Materials and Methods

Hindu College, Guntur campus is divided into 07 zones and in each zone five locations are selected and from each location soil samples are collected, near roots where most of the microbial activity is concentrated. Soil samples (approximately 5g) are collected with clean, dry and sterile polythene bags along with sterile spatula. The collected samples are brought to the laboratory and preserved for further studies. The soil samples are collected from the month of January 2022 to March 2022 in Hindu college campus at various locations. The soil samples collected from seven different zones of Hindu college campus are mentioned in Table 1.

Figure 1. Collection of Soil Sample

Table 1. Soil collected from different zones of Hindu college

S. No.	Zone
1.	Parking Area
2.	Main Entrance
3.	Badminton Court
4.	Cricket Ground
5.	Saraswathi Vanam
6.	Flower Garden
7.	Nakshatra Vanam

2.1. Preparation of soil sample and microbial culture

1 gram of collected soil samples are diluted with 09 ml of sterile distilled water. 0.1ml of suspension is added to sterile petri plates in triplicates containing sterile Potato Dextrose Agar (PDA) medium (fungal medium). The plates are incubated at temperature of 37^0C for 3-5 days. A greater number of species are isolated and most of the fungus appear as heavily sporulated on PDA (potato dextrose plates) plates. Pure culturing is done using test tubes containing fresh agar slants of PDA medium. The test tubes are stored in refrigerator. When inoculums are transferred into petri plates containing fungal nutrient media cells are not separated from each other. Therefore, mixed colonies are developed. Hence isolation of pure culture from mixed colonies is rather difficult. Therefore, spread plate technique is employed for pure culturing.

2.2. Potato dextrose agar [PDA] medium

Potato dextrose agar is a general medium for yeasts and moulds that can be supplemented with acid or antibiotics to inhibit bacterial growth. PDA can be used for growing clinically significant yeasts and moulds.The nutritionally rich base [potato infusion] encourages mould sporulation and pigment production in some dermatophytes.

Uses of Potato Dextrose Agar [PDA]

Potato dextrose agar with chloramphenicol is recommended for the selective cultivation of fungi from mixed samples. It may also be used for cultivation of yeasts and moulds from clinical specimens.

Composition of Potato Dextrose Agar [PDA]

Potato infusion - 200gm

Dextrose - 20gm

Agar - 20gm

Distilled water - 1liter

pH - 5.6 ± 0.2

Figure 2. PDA Medium Planting and Sampling

2.3. Staining of fungi

The fungal propagules are either hyaline (colourless) or different colours. The hyaline mycelia / spores / conidia and cytoplasm can be stained by using Lactophenol (in unavailability, Crystal violet). Cotton blue stains cytoplasm and results in light blue background. Lactophenol acts as a cleaning agent. The stained specimens are observed under the light microscope for identification and microphotograph is taken under 10x × 45x magnification.

2.4. The effect of Lactophenol Cotton Blue

Lactophenol Cotton Blue (LPCB) is a stain used for making semipermanent microscopicpreparation of fungi. The LPCB stain has three following components.

- Phenol: kills any organism.
- Lactic acid: preserves fungal structures.
- Cotton blue: stains the chitin and cellulose of the fungal cell wall intensely blue.

Figure 3. Staining

2.5. Identification of Fungi

The isolated fungus is identified to the genus level and to the species when possible on the basis of macro morphological (the colonies are examined for slow or rapid growth) topographical (flat, heaped, regularly or irregularly folded), textural (yeast like, powdery, granular, velvety or cottony), surface pigmentation and reverse pigmentation and micro morphological (Hyphae, macro conidia, micro conidia, chlamydospores and other special fungal structure) characteristics using suitable media, slide cultures and the most updated keys for identifications. The identified fungus is confirmed with microbial expert.

3. Results

The study is aimed for the isolation of soil fungi from different locations of Hindu college campus, Guntur and grown invitro during the period of January 2022 to March 2022. Isolated fungi are identified with help of standard books. From the fungal isolates the species belonging to the genera *Aspergillus* and *Mucor* are dominant. The identified soil fungus is *Aspergillus niger, Aspergillus fumigatus, Penicillium chrysogenum, Colletotrichum*

gloeosporioides, Mucor species like Rhizopus stolonifer, Rhizopus oryzae, Cladophialophora species etc. Among the identified species the *Keratinophilic* fungi *Aspergillus niger* are found in maximum numbers in the campus and followed by Mucor species. Broad soil type and slit or clay is defined as largest to smallest of particle size. These particles pack loosely, and plant roots are particular habitats for microorganisms, often in biofilms. Soil also contains plants, animal carcasses and man-made materials. A gram of garden soil may contain around one million fungi such as yeasts, and moulds. Fungi have no chlorophyll and are not able to photosynthesize. They cannot use atmospheric carbon dioxide as a source of carbon; therefore, they are chemoheterotrophic. Many fungi are parasitic, often causing disease to their living host plant although some have beneficial relationship with plants. In terms of soil and humus creation, the most important fungi tend to be saprotrophic. Fungi is present where adequate moisture, temperature and organic substrates are available. Although we normally think of fungi as growing in warm moist forest, many species occur in habitats that are cold, periodically arid, or otherwise seemingly inhospitable. It is important to recognize the conditions for growth and reproduction vary widely with fungal species. Diversity of most groups of fungi tend to increase in tropical regions, but detailed studies are only in their infancy. From the mycelia, the fungi is able to throw its fruiting, the visible part above the soils (e.g. mushrooms, toadstools, puffballs), may contain millions of spores. Fungi have 40–55% carbon using efficiency so they store and recycle more carbon (C) compared to bacteria.

Figure 4. *Aspergillus* Colonies

Figure 5. Observing *Aspergillus Niger* Under Microscope

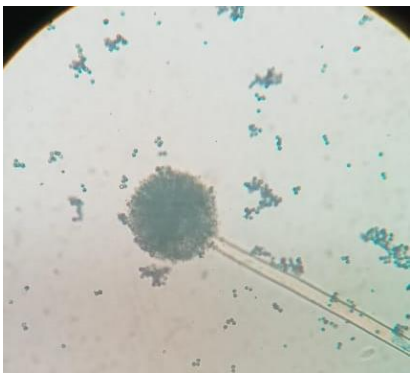

Figure 6. *Aspergillus Niger* Under Microscope

4. Conclusion

Aspergillus species have great commercial importance. These are useful in production of organic acid like citric acid, gluconic acid, etc. These are involved in production of antibiotics like Proliferin, Fumigalin. Amylase

production can be done by *A.niger* and *A.oryzae*. Also helps in bioassay of soil for tracing out the elements like copper and arsenic by A.niger and *A.virens*.

Applications

(a) *Aspergillus niger* is cultured for the industrial production process of various substances. Various strains of *Aspergillus niger* are used in the industrial preparation of the citric acid and gluconic acid. These are acceptable for day-to-day intake by the World Health Organization. *Aspergillus niger* fermentation is GRAS (Generally Recognized As Safe) by the US food and drug administration under the Federal Food, Drug, and cosmetic act.

(b) *Aspergillus niger* inoculation increases seedling root growth of lettuce, pepper, scarlet eggplant, watermelon, and tomato.

(c) Production of many useful enzymes occurs with the use of industrial fermentation of *Aspergillus niger* (in food industry)

(d) *Aspergillus niger* is also useful for the extraction of the enzyme, glucose oxidase, we use it in the design of glucose biosensors.

(e) *Aspergillus niger*, is the source of anti-cancer compounds such as Nigerapyrone-B, Asnipyrone-A, Nigerasterol-A and L-asparginase.

Declarations

Source of Funding

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Competing Interests Statement

Authors have declared no competing interests.

Consent for Publication

The authors declare that they consented to the publication of this study.

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